1,018,647

PATENT SPECIFICATION

DRAWINGS ATTACHED

1,018,647

Date of Application and filing Complete Specification: Dec. 11, 1963.
No. 48942/63.
Application made in Japan (No. 56118) on Dec. 20, 1962.
Complete Specification Published: Jan. 26, 1966.

Index at acceptance:—A5 B(1C, 1H, 1J, 2C, 2H, 2J)

Int. Cl.:—A 61 k 3/08

COMPLETE SPECIFICATION

Method for Stabilizing a Hemostatic Preparation

We, CHUGAI SEIYAKU KABUSHIKI KAISHA, a joint stock company organized under the laws of Japan, of No. 3, Nihaonbashi Honcho 3-Chome, Chuo-ku, Tokyo, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a method for stabilizing an aqueous dispersion of hemostatic phospholipids, for example lipid-thromboplastin.

Hitherto, with a view to preventing the rancidity of fatty substances such as phospholipids, we have added a lipid-soluble antioxidant such as, for example, butylated hydroxy anisole (B H A), nordihydroguaiaretic acid (N D G A), ethyl pyrotocatechuate, or α-tocopherol, to said fatty substances. However, the addition of said antioxidants to the aqueous dispersion of the phospholipid is made with difficulty because said antioxidants are lipid-soluble but water-insoluble. Further, most of said antioxidants are not completely satisfactory because of their liability to develop a reddish colour on exposure to light even in very dilute aqueous solution.

We have previously proposed to provide a method for the stabilization of the hemostatic preparations by the addition of ascorbic acid. The hemostatic activity of such stabilized preparation does not decrease very much even upon standing at room temperature. However, ascorbic acid itself can be oxidized to form a yellow substance and the ascorbic acid-containing preparation will then turn yellow. It has been observed that in particular, if a different substance such as amino acid is also present in the dispersion, the discolouration could not be prevented.

The present invention has resulted from our attempts to obtain a stable aqueous dispersion of hemostatic phospholipid which can be kept for a long period of time at room temperature, without decreasing its hemostatic activity and without colouring even in the presence of substances which can be present in the hemostatic preparation, for example, amino acids, vitamins and the like and which are able to be administered both intravenously and intramuscularly.

According to the present invention, a method for stabilizing an aqueous dispersion of a hemostatic phospholipid for example lipid thromboplastin is provided in which one or more water-soluble reducing agents which contain sulphur in the reactive (or reductive) group in the molecule is added as a stabilizer to an aqueous dispersion of the phospholip.

Examples of these sulphur-containing reducing agents which may be employed are sulfites, bisulfites, metabisulfites, thiosulfates, sulfoxylates, α-mono-thioglycerol.

An aqueous dispersion of hemostatic phospholipid in this invention means such a one as is obtained by dispersing hemostatic phospholipid, for example lipid-thromboplastin in water or water containing sodium chloride, amino acid or a vitamin.

The water-soluble reducing agents having sulfur atom or atoms in the molecule which are employed in this invention as the stabilizers and their stabilizing effects will be specifically explained. Firstly, as specific examples of the stabilizers, sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), potassium meta-bisulfite ($K_2S_2O_5$), sodium thiosulfate ($Na_2S_2O_3$), rongalite

$$(HOH_2C.SO_2Na.2H_2O),$$

α-monothio-glycerol

$$HSCH_2.CH(OH).CH_2OH)$$

may be mentioned. All these stabilizers, when added to the aqueous dispersion of hemostatic phospholipid act to consume oxygen dissolved in water by being oxidized themselves and

prevent the hemostatic substances from autoxidation and polymerization caused by the action of oxygen.

The hemostatic preparation may be advantageously prepared by the introduction of an inert gas such as nitrogen into the water in the process for dispersing the hemostatic substance in water. By this procedure, oxygen dissolved in the water may be replaced by the inert gas, whereupon the hemostatic substance may be prevented from being contacted with oxygen and the amount of the stabilizer to be employed may be reduced.

By means of the present invention, it is possible to obtain a hemostatic agent which is free from discolouring and from decrease of the activity and which can be preserved for long periods at room temperature even in the presence of substances which can be present in the hemostatic preparation, for example amino acids, vitamins and the like. As amino acid, ε-aminocaproic acid, for example, which is commonly well known as an antifibrinolytic agent may be incorporated with the hemostatic substance. Further, the hemostatic preparation of this invention can be adjusted to pH 6.0—8.0 at will, so that it may be administered intravenously and intramuscularly.

Experimental method.

A solution of 0.025 mg/cc of thromboplastin in the form of phosphatide was prepared. To the solution (A) the following five compounds: ascorbic acid (B), potassium meta-bisulfite (C), L-mono-thioglycerol (D), sodium bisulfite (E) and rongalite (F) were separately incorporated to give 10 mg/cc solutions. 5 ml. of each of these five samples were treated as follows:

(1) Heated at 100° C for 10 hours
(2) Heated at 80° C for one week
(3) Left to stand for one week under irradiation by ultra-violet ray.

Thereafter the degree of colouration against a standard white plate was measured by means of a photoelectric reflection meter (Hitachi Seisakusho). The value of the sample of freshly prepared C was taken as 100 as a control value and the comparative values of each of the other samples were obtained based on the value of C, as follows:

COMPARISON OF THE DEGREE OF COLOURATION BASED ON THE RELATIVE WHITENESS

Stabilizer / Conditions	A	B	C	D	E	F
Freshly prepared	80.5	92.3	100.0	88.9	99.2	99.8
After standing at 100° C. for 10 hrs.	39.4	45.7	108.3	61.6	96.7	102.3
After standing at 80° C. for 1 week	38.9	46.2	87.7	67.7	82.3	90.6
After standing at 1 week under ultra violet rays	63.3	68.9	105.0	87.7	94.1	95.4

Note: A and B relate to the hitherto known method. C, D, E and F relate to the method of the present invention.

It is clear from the above table that the hemostatic preparation stabilized by the method of this invention keeps an extraordinarily high degree of relative whiteness and the stabilizers used present an excellent effect in the prevention of discolouration.

The stabilizing effect with the lapse of time of hemostatic action in the case when potassium meta-bisulfite is incorporated with lipid-thromboplastin. The results obtained with potassium meta-bisulfite added in concentration of 0.5 percent are illustrated by the graph in the accompanying drawing.

The blood platelet activity of the sample was measured by means of thromboplastin-forming tests described by Biggs and Douglas: J. Clin. Path. June 23, 1953, after standing for 3—20 days at various temperature conditions. In the accompanying drawings, solid lines represent activity as a percentage of the original activity in the case when potassium meta-bisulfite is incorporated, and broken lines represent the activity as a percentage of the original activity in the case when not incorporated. In the former case excellent stabilizing effects of hemostatic action

are observed upon standing at every temperature, whereas in the latter case the activities fall rapidly at every temperature.

Similar results have been obtained with the other stabilizers mentioned above.

EXAMPLE 1.

220 mg of lipid-thromboplastin were added to 44 ml of physiological solution containing 35.2 mg of nonionic surface active agent polyoxyethylene sorbitan manolaurate (TWEEN—20) (produced by Atlas Powder Co. U.S.A. "Tween" is a Registered Trade Mark) while stirring and with the introduction of nitrogen gas thereinto. After finely dispersing lipid-thromboplastin, 44 mg of potassium metabisulfite were added. The dispersion was then adjusted to pH 7.0 and filtered. 5 cc of each dispersion thus obtained was sealed in a brown ampoule, each ampoule having been flushed with nitrogen prior to sealing, and then sterilized by heating at 100° C for 40 minutes to obtain a product.

EXAMPLE 2.

24 mg of nonionic surface active agent polyoxyethylene sorbitan monolaurate (TWEEN—20) and 2 g of ε-aminocaproic acid were dissolved in distilled water to make 40 ml of solution. To the solution, while stirring and introducing nitrogen gas therein, a solution of 500 mg of lipid-thromboplastin in 6 cc of ether were added and finely dispersed volatilizing off ether, by heating to 50° C on a water bath. After cooling to room temperature, 100 mg of potassium meta-bisulfite were added to dissolve, the dispersion adjusted to pH 7.5 and filtered. Each 2 cc of the dispersion thus obtained was sealed in a brown ampoule, each ampoule having been flushed with nitrogen prior to sealing and then sterilized.

EXAMPLE 3.

Instead of 44 mg of potassium metabisulfite 44 mg of sodium bisulfite were added as in Example 1.

EXAMPLE 4.

Instead of 100 mg of potassium metabisulfite 100 mg of sodium bisulfite were added as in Example 2.

EXAMPLE 5.

Instead of 44 mg of potassium metabisulfite 44 mg of sodium sulfite were added as in Example 1.

EXAMPLE 6.

Instead of 100 mg of potassium metabisulfite 100 mg of sodium sulfite were added as in Example 2.

EXAMPLE 7.

Instead of 100 mg of potassium metabisulfite 100 mg of rongalite were added as in Example 2.

EXAMPLE 8.

Instead of 100 mg of potassium metabisulfite 100 mg of α-monothioglycerol were added as in Example 2.

EXAMPLE 9.

Instead of 100 mg of potassium metabisulfite 100 mg of sodium thiosulfate were added as in Example 2.

The preparations above thus obtained do not suffer any undesirable changes such as colouration and the deterioration of hemostatic activity but are very stable and can be administered intravenously and intramuscularly.

WHAT WE CLAIM IS:—

1. Method for stabilizing an aqueous dispersion of a hemostatic phospholipid which comprises adding, as a stabilizer, a water-soluble reducing agent which contains sulphur in the reactive (or reductive) group in the molecule to an aqueous dispersion of a hemostatic substance consisting of phospholipid, for example lipid-thromboplastin.

2. Method for stabilizing a hemostatic preparation which comprises adding, as a stabilizer, a water-soluble reducing agent which contains sulphur in the reactive (or reductive) group in the molecule selected from sodium sulfite, sodium bisulfite, potassium meta-bisulfite, sodium thiosulfate, rongalite and α-monothioglycerol to an aqueous dispersion of hemostatic substance consisting of phospholipid.

3. Method for stabilizing a hemostatic preparation according to Claim 2 which further comprises adjusting the aqueous dispersion obtained to pH 6.0—8.0.

4. A stabilized hemostatic preparation prepared by the method claimed in any one of the preceding Claims 1 to 3.

5. Method for stabilizing a hemostatic preparation substantially as set forth in any one of Examples 1 to 9 herein.

For the Applicants,
F. J. CLEVELAND & COMPANY,
Chartered Patent Agents,
Lincoln's Inn Chambers,
40/43, Chancery Lane, London, W.C.2.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press (Leamington) Ltd.—1966. Published by The Patent Office, 25 Southampton Buildings, London, W.C.2, from which copies may be obtained.

1018647 COMPLETE SPECIFICATION

1 SHEET

This drawing is a reproduction of the Original on a reduced scale